EVALUATION OF SERUM LEVELS OF OSTEOPONTIN AND BONE SIALOPROTEIN IN FEMALE PATIENTS WITH TENDINOPATHY

KADIN TENDİNOPATİ HASTALARINDA SERUM OSTEOPONTİN VE BONE SİALOPROTEİN DÜZEYLERİNİN DEĞERLENDİRİLMESİ

TENDINOPATHY AND SIBLING FAMILY

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This study was previously available as poster presentations in World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (14-17 April 2016) at Malaga/Spain.

Öz

Amaç: Tendinopati hastalık patogenezi kesin olarak ortaya konulamamıştır. Küçük integrin bağlayıcı ligand N-bağlı glikoprotein (SIBLING) ailesi, osteopontin (OPN) ve kemik sialoprotein (BSP) moleküllerini de içeren kollajen-dışı protein ailesidir. Önceki çalışmalarda, SIBLING ailesi moleküllerinin mineralize dokular ile sınırlı olduğu düşünülmüş ancak son yapılan çalışmalarda daha geniş dağılımı olduğu ve non-mineralize dokularda da eksprese olduğu gösterilmiştir. Çalışmamızda, tendinopati hastalığı ile serum OPN ve BSP arasındaki ilişkiyi araştırmayı amaçladık. Gereç ve Yöntem: Bu prospektif çalışmaya, 39 kadın tendinopati hastası ve 39 sağlıklı kadın dahil edilmiştir. Serum OPN ve BSP seviyeleri, enzim-ilişkili immunosorbent yöntemi ile ölçüldü. Ayrıca vücut kitle indeksi, eritrosit sedimentasyon hızı (ESR), beyaz küre sayısı (WBC) ve nötrofil-lenfosit oranı (NLR) ölçüldü. Bulgular: İki grup arasında serum BSP düzeyleri arasında fark saptanmadı (41.83 ± 52.03 vs. 53.64 ± 53.06 ng/mL, p=0.276). Hasta ve kontrol grup arasında serum OPN düzeyleri açısından fark bulunamadı (57.37 ± 21.61 vs. 77.72 ± 72.14 ng/mL, p=0.363). İki grup arasında WBC, NLR ve ESR değerleri açısından fark bulunamadı (p=0.897, p=0.795, p=0.405 geriye dönük olarak). Hasta grubunda, serum BSP seviyeleri ile WBC, ESR ve NLR değerleri arasında korelasyon tespit edilemedi. Tendinopati hastalarında serum OPN seviyeleri ile NLR değerleri arasında negatif korelasyon saptandı. Tartışma: Bu çalışma sonuçlarına göre, tendinopati hastalığı patogenezinde OPN ve BSP molekülleri yer almamaktadır.

Anahtar Kelimeler

Tendinopati; Kemik sialoprotein; Osteopontin; SIBLING Ailesi; BSP; OPN

Abstract

Aim: The pathogenesis of tendinopathy remains unclear. Small integrin-binding ligand N-linked glycoproteins (SIBLING), a family of non-collagenous proteins including osteopontin (OPN) and bone sialoprotein (BSP), were initially thought to be limited to mineralized tissue, but recent studies have reported that they are more widely distributed and are expressed in non-mineralized tissues. The aim of this study was to investigate relationships between serum OPN and BSP levels and tendinopathy disease. Material and Method: 39 female tendinopathy patients and 39 female healthy volunteers were recruited for this prospective observational study. Serum OPN and BSP levels were measured using enzyme-linked immunosorbent assay. We also measured body mass index and erythrocyte sedimentation rate (ESR), white blood cells (WBC), and neutrophil-lymphocyte ratio (NLR). Results: There were no significant differences in serum BSP levels between the two groups (41.83 ± 52.03 vs. 53.64 ± 53.06 ng/mL, p=0.276). There were also no significant differences in serum OPN levels between the two groups (57.37 ± 21.61 vs. 77.72 ± 72.14 ng/mL, p=0.363). There were no significant differences in WBC, NLR, and ESR values between the two groups (p=0.897, p=0.795, p=0.405, respectively). There was no correlation between serum BSP levels and OPN, WBC, NLR, and ESR levels in the patient group. Patients with tendinopathy had a negative correlation between serum OPN levels and NLR levels. Discussion: The results of this study have indicated that BSP and OPN levels are not involved in the pathogenesis of tendinopathy.

Keywords

Tendinopathy; Bone Sialoprotein; Osteopontin; SIBLING Family

DOI: 10.4328/JCAM.4854 Received: 03.11.2016 Accepted: 07.12.2016 Printed: 01.07.2017 J Clin Anal Med 2017;8(4): 271-4
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Introduction

Tendinopathy is a condition characterized by a change in the inflammatory response of tendons to stress, degeneration of the tendons, and tearing of the tendon fibrils. This condition can be caused by excessive and repeated mechanical load. It is thought that this can cause disruption of the integrity of the matrix and degeneration of the tendons by activating oxidative stress and apoptotic processes [1, 2]. In affected tendons, thin and disorganized collagen fibrils, increased collagen and matrix protein, increased active tenosynovitis, and revascularization of the tendons can be seen [3]. The etiology and pathogenesis of the disease are not fully understood, but many risk factors such as obesity, inflammation, trauma, hypoxia, and drugs such as fluoroquinolone have been recognized [2-5].

Small integrin-binding ligand N-linked glycoproteins (SIBLINGs) are a family of non-collagenous proteins consisting of five members: osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein (DMP1), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE). SIBLING family members were initially thought to be limited to mineralized tissue, i.e. bones and teeth, but studies have revealed that they are more widely distributed and are also expressed in nonmineralized normal tissues such as salivary gland and kidney [6-8]. A recent study in 2016 showed that OPN and BSP-4 were present in enthesis; the study suggested that BSP plays a regulatory role in enthesis and may be a useful therapeutic molecule in the reattachment of tendons and ligaments to bone [9].

Musculoskeletal tissue cells, including osteoblasts, chondrocytes, myoblasts, and skin and tendon fibroblasts, share the common stem cells, differentiated mesenchymal stem cells [10,11]. Thus, Mori et al. have suggested that the musculoskeletal tissues should be categorized as the same functional unit developed from the mesenchymal stem cells [12]. This theoretical background led us to hypothesize that OPN and BSP could be involved in tendinopathy pathogenesis.

The aim of this study was to compare bone sialoprotein and osteopontin and inflammatory parameters [erythrocyte sedimentation rate (ESR), white blood cell (WBC), neutrophil-lymphocyte ratio (NLR)] levels between tendinopathy and the control group and to present new evidence related to pathogenesis tendinopathy disease.

Material and Method

Study population:

This pilot, prospective, case-control study was conducted at Dicle University, Medical Faculty Hospital, Diyarbakır, Turkey between January 2016 and April 2016. Tendinopathy diagnosis was based on physical examination and radiological evaluation. 39 female tendinopathy patients aged 24-34 years were included in the study. 39 female healthy volunteers, with normal radiological examinations and clinical histories and examinations, who visited the hospital for routine physical examinations were enrolled as controls. We included only female patients to homogenize the sample, as there are differences between women and men that couldinfluence BSP and OPN levels. Controls were matched in terms of age and body mass index (BMI) with the patients. The BMI is defined as the body mass (weight) divided by the square of the body height, and is universally expressed in units of kg/m2. Clinical information including age, course of disease, and biochemical analyses (whole blood count, erythrocyte sedimentation rate) were obtained from the hospital data system.

Participants with a history of smoking, acute or chronic infectious diseases, cardiovascular diseases, cerebrovascular disease, diabetes mellitus, obesity (BMI >30), abnormal liver or renal function tests, malignancies, musculoskeletal surgery or injury within the previous two months, other musculoskeletal disease (rheumatoid arthritis etc.), autoimmune, endocrine, or metabolic disorders, cancers, pregnancy, thrombosis, and liver or chronic renal insufficiency were excluded from the study.

The study was approved by the Research Ethics Committee of Dicle University Medical School and was performed in accordance with the ethical standards stated in the 1964 Declaration of Helsinki. Written informed consent was obtained from all patients and healthy volunteers prior to their participation in this study.

Standard tubes with a constant amount of ethylenediaminetetraacetic acid (EDTA) were used for the whole blood count. All blood samples were studied within one hour of sampling. The whole blood count analyses, based on the technique of laser flow cytometry scattergrams, were performed in the central laboratory of our institution using the same analyzer (Medonic CA-620, Sweden) which is routinely checked every day. Whole blood count parameters of participants were recorded from the same computerized database. ESR was determined using an automated Westergren method (Sedimat 15, LP Italiana, Italy).

Measurement of OPN and BSP

Blood samples were obtained from the antecubital vein of all participants after overnight fasting. After clotting, blood samples were centrifuged at 2000 × g for 10 min, serum was separated, and aliquots were stored at −80°C until examination. Se¬rum OPN and BSP concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit (SunRedbio; Shanghai, China) according to the manufacturer's instructions. The samples were processed as recommended by the kit and run in random for the ELISA. The colour intensities were measured by a plate reader (DAR 800 microplate reader, Chemtron Pte Ltd, Singapore) with a measuring filter of 450 nm. The results were expressed as nanograms per milliliter (ng/mL). The standard curve ranged from 20 ng/mL to 640 ng/mL of OPN and the standard curve showed a direct relation between optical density and OPN concentration. The standard curve of ELISA for BSP ranged from 5 ng/mL to 160 ng/mL and the standard curve showed a direct relation between optical density and BSP concentration. The sensitivity of the BSP and OPN commercial kit were 0.435 ng/mL and 2.045 ng/mL, respectively. The intra-assay coefficients of variation of the assays were <10%. Assay ranges were 0.5-150 ng/mL for BSP and 5-600 ng/mL for OPN.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 software (Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Wilk test. Data are expressed as mean±standard deviation. Student's t-test for independent samples was used to analyze and in cases of normal distribution, Mann-Whitney's U test was used to compare the groups.

The correlations between the variable pairs were analyzed using Spearman's correlation test. Differences between groups were significant when p< 0.05.

Results

A total of 78 female patients (39 tendinopathy and 39 controls) were included in the study. Baseline clinical characteristics are shown in Table 1. There were no significant differences between the patient group with tendinopathy and healthy controls in terms of age and BMI (p=0.162, p=0.362, respectively).

WBC values were 7.18 ± 1.49 x10³/mm³in patients with tendinopathy vs. 7.30 ± 1.48 x10³/mm³ in the control group. Mean NLR levels were 2.12 ± 0.75 in patients with tendinopathy vs. 2.18 ± 0.47 in the control group. ESR values were 12.1 ± 9.8 mm/h in patients group and 10.9 ± 3.4 mm/h in controls group. There were no significant differences in WBC, NLR, and ESR values between the two groups (p=0.897, p=0.795, p=0.405, respectively) (Table 1).

Table 1. Baseline clinical characteristics of tendinopathy patients and the controls

	Tendinopathy patients (n=39)	Controls (n=39)	p value
Age	29 ± 5	27 ± 5	0.162
BMI (kg/m2)	23.32 ± 1.57	23.42 ± 2.76	0.362
WBC (x10³/mm³)	7.18 ± 1.49	7.30 ± 1.48	0.897
NLR	2.12 ± 0.75	2.18 ± 0.47	0.795
ESR (mm/h)	12.1 ± 9.8	10.9 ± 3.4	0.405

Abbreviations: ESR: Erythrocyte sedimentation rate; WBC: White blood cell; NLR: Neutrophil to lymphocyte ratio; BMI: Body mass index

Mean BSP values were 41.83 ± 52.03 ng/mL in patients with tendinopathy vs. 53.64 ± 53.06 ng/mL in the control group. Mean OPN values were 57.37 ± 21.61 ng/mL in patients with tendinopathy vs. 77.72 ± 72.14 ng/mL in the control group. There were no significant differences in serum BSP and serum OPN levels between the two groups (p=0.276, p=0.363, respectively) (Table 2).

Table 2. Serum levels of BSP and OPN in tendinopathy patients and the controls

	Tendinopathy patients (n=39)	Controls (n=39)	p value
BSP (ng/mL)	41.83 ± 52.03	53.64 ± 53.06	0.276
OPN (ng/mL)	57.37 ± 21.61	77.72 ± 72.14	0.363

Abbreviations: BSP: Bone sialoprotein; OPN: Osteopontin

There was no correlation between serum BSP levels and OPN, WBC, NLR, and ESR levels in the patient group (Table 3). Patients with tendinopathy had a negative correlation between serum OPN levels and NLR levels, but no significant correlation was found between serum OPN levels and WBC and ESR levels (Table 4).

Discussion

Tendinopathy is a painful condition that occurs in and around tendons in response to overuse. Various intrinsic and extrinsic risk factors have been identified, but the molecular pathogenesis and etiology of the disease remains unclear and unknown.

Table 3. Relationship between BSP and OPN, WBC, NLR and ESR in tendinopathy patients

		OPN	WBC	NLR	ESR
BSP	r value	0.179	0.112	-0.154	0.021
	p value	0.275	0.497	0.351	0.898

Abbreviations: ESR: Erythrocyte sedimentation rate; WBC: White blood cell; NLR: Neutrophil to lymphocyte ratio; BSP: Bone sialoprotein; OPN: Osteopontin

Table 4. Relationship between OPN and BSP, WBC, NLR and ESR in tendinopathy patients

		BSP	WBC	NLR	ESR
OPN	r value	0.179	0.044	-0.337	-0.156
	p value	0.275	0.789	0.036	0.342

Abbreviations: ESR: Erythrocyte sedimentation rate; WBC: White blood cell; NLR: Neutrophil to lymphocyte ratio; BSP: Bone sialoprotein; OPN: Osteopontin

To our knowledge, this is the first study to reportserum BSP and OPN levels in tendinopathy patients; we demonstrated that OPN and BSP levels did not significantly differ in the sera of tendinopathy patients, when compared to a healthy control group.

The SIBLING protein family has been defined based on the common structural, biochemical, and genetic features of its members [13]. They share common exon-intron features, are located on the same human chromosome (4q21), contain an Arg-Gly-Asp (RGD) integrin binding site that mediates cell attachment/signaling, and undergo similar post-translational modifications such as phosphorylation and N-glycosylation [6,13].

Bone sialoprotein is an acidic phosphorylated glycoprotein containing RGD cell attachment sequence that belongs to the SIBLING family of proteins. BSP is a multifunctional protein and is synthesized by osteoblasts, osteoclasts, and other skeleton-associated cell types [14]. BSP plays a crucial role in the process of bone mineralization, resorption, remodeling, and repair [6]. Several studies have revealed that BSP is a promoter for the initial formation of hydroxyapatite crystals in mineralized tissue and an early marker of osteogenic differentiation [13, 15, 16]. BSP is overexpressed in osteotrophic cancer tissues, including breast, colon, prostate, lung, and thyroid cancers and is involved in cancer cell growth, adhesion, proliferation and migration, metastasis, and angiogenesis [6, 17, 18). Also, Bellahcene et al. demonstrated that BSP mediates human endothelial cell attachment and migration through the interaction of its RGD domain with endothelial cell integrin receptors and might be an important factor for the angiogenesis process in bone formation and tumour growth and metastasis [19]. In a recent 2016 study, Marinovich et al. reported that BSP presents in the mouse enthesis and BSP plays a regulatory role in this structure [9]. In this study, we analyzed serum BSP levels in tendinopathy patients and we showed that no significant differences were found in serum BSP levels between tendinopathy patients and controls. Our results indicate that BSP is not related to the pathogenesis of tendinopathy.

Osteopontin (OPN), also known as secreted phosphoprotein (Spp1) or early T lymphocyte activation-1 (Eta-1), is a multifunctional cytokine, adhesion protein, and a member of the SIBLING ligand family. OPN is expressed in various cell types and tissues such as osteoblasts, osteocytes, chondrocytes, smooth muscle cells, skeletal muscle, T cells, macrophages and dendritic cells, endothelial cells, fibroblasts, and hepatocytes [20].

OPN is involved in a wide spectrum of physiological and pathological processes such as biomineralization, tissue remodeling, wound healing, tumorigenesis, fibrosis, cellular immunity, and inflammation [20, 21]. Studies have shown that OPN is associated with the pathogenesis of autoimmune disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, atherosclerosis, chronic obstructive pulmonary disease, Crohn's disease, liver diseases, cancers, obesity, and diabetes [20-22]. Takeuchi et al. showed that OPN is involved in the process of calcification of rotator cuff tendons [23]. Mori et al. revealed that OPN plays a crucial role in regulating tendon remodeling induced by stress deprivation and that OPN may act as a transducer of mechanical stress to the tendon fibroblasts [12]. Recently, Marinovich et al. showed that OPN presents in the mouse enthesis [9]. In this study, we examined serum OPN levels in tendinopathy patients and we showed that no significant differences were found in serum OPN levels between tendinopathy patients and controls. Our results indicate that OPN is not involved in the pathogenesis of tendinopathy.

The role of inflammation in the development and progression of tendinopathy has been investigated in many studies, but it is still uncertain and controversial. With results from newly-developed immunohistochemical techniques and genetic studies, it is now thought that inflammation plays a role in the pathogenesis of the disease [3]. We investigated the relationship between serum OPN and BSP levels and inflammatory markers including NLR and ESR in patients with tendinopathy. We did not find any correlations between BSP levels and inflammatory markers. But we found a negative correlation between OPN and NLR levels in the patient group. This is consistent with previous studies. Multiple studies have demonstrated that OPN is expressed by immune system cells such as neutrophils and macrophages and was found to act as a proinflammatory cytokine and to play an important role in inflammation and regulation of tissue repair [20]. OPN induces neutrophil migration and serves as a potent neutrophil chemoattractant [24]. In previous studies, the relationship between OPN and NLR in liver cancer was investigated and it has been suggested that the combination of OPN and NLR may have a stronger prognostic value in primary liver cancer [25]. In future studies, the relationship between OPN and NLR should be analyzed in inflammatory conditions and tendon related diseases.

We must acknowledge some limitations of this study. The sample size of our study was small. Moreover, in our study, patient and control groups consisted of the same gender (female). Previous studies have shown that gender differences could affect BSP and OPN levels so we included only female patients to homogenize the sample.Thus, the subjects are not representative of the general population, but instead representative only of the female gender. In future studies the levels of BSP and OPN should be analyzed in the general population.

This pilot study demonstrates that serum OPN and serum BSP levels did not differ in the tendinopathy patients group and this indicates that BSP and OPN are not involved in tendinopathy pathogenesis. Further large-scale and more detailed prospective studies of the relationship between small integrin-binding ligand N-glycoproteins family members and tendinopathy are needed.

Acknowledgement:

The authors report no declarations of conflicts of interest. The work was not supported or funded by any company.

Competing interests

The authors declare that they have no competing interests.

References

1. Jiang C, Shao L, Wang Q, Dong Y. Repetitive mechanical stretching modulates transforming growth factor-β induced collagen synthesis and apoptosis in human patellar tendon fibroblasts. Biochem Cell Biol 2012;90(5):667-74.
2. Benson RT, McDonnell SM, Knowles HJ, Rees JL, Carr AJ, Hulley PA. Tendinopathy and tears of the rotator cuff are associated with hypoxia and apoptosis. J Bone Joint Surg Br 2010;92(3):448-53.
3. Dean BJ, Gettings P, Dakin SG, Carr AJ. Are inflammatory cells increased in painful human tendinopathy? A systematic review. Br J Sports Med 2016;50(4):216-20.
4. Franceschi F, Papalia R, Paciotti M, Franceschetti E, Di Martino A, Maffulli N et al. Obesity as a risk factor for tendinopathy: a systematic review. Int J Endocrinol 2014;2014:670262.
5. Kaleagasioglu F, Olcay E. Fluoroquinolone-induced tendinopathy: etiology and preventive measures. Tohoku J Exp Med 2012;226(4):251-8.
6. Bellahcène A, Castronovo V, Ogbureke KU, Fisher LW, Fedarko NS. Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. Nat Rev Cancer 2008;8(3):212-26.
7. Ogbureke KU, Fisher LW. Expression of SIBLINGs and their partner MMPs in salivary glands. J Dent Res 2004;83(9):664-70.
8. Ogbureke KU, Fisher LW. Renal expression of SIBLING proteins and their partner matrix metalloproteinases (MMPs). Kidney Int 2005;68(1):155-66.
9. Marinovich R, Soenjaya Y, Wallace GQ, Zuskov A, Dunkman A, Foster BL et al. The role of bone sialoprotein in the tendon-bone insertion. Matrix Biol 2016;52-54:325-38.
10. Salingcarnboriboon R, Yoshitake H, Tsuji K, Obinata M, Amagasa T, Nifuji A et al. Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property. Exp Cell Res 2003;287(2):289-300.
11. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg Am 2005;87(1):187-202.
12. Mori N, Majima T, Iwasaki N, Kon S, Miyakawa K, Kimura C et al. The role of osteopontin in tendon tissue remodeling after denervation-induced mechanical stress deprivation. Matrix Biol 2007;26(1):42-53.
13. Fisher LW, Torchia DA, Fohr B, Young MF, Fedarko NS. Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. Biochem Biophys Res Commun 2001;280(2):460-5.
14. Gordon JA, Tye CE, Sampaio AV, Underhill TM, Hunter GK, Goldberg HA. Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization in vitro. Bone 2007;41(3):462-73.
15. Staines KA, MacRae VE, Farquharson C. The importance of the SIBLING family of proteins on skeletal mineralisation and bone remodelling. J Endocrinol 2012;214(3):241-55.
16. Wang J, Zhou HY, Salih E, Xu L, Wunderlich L, Gu X et al. Site-specific in vivo calcification and osteogenesis stimulated by bone sialoprotein. Calcif Tissue Int 2006;79(3):179-89.
17. Fisher LW, Jain A, Tayback M, Fedarko NS. Small integrin binding ligand N-linked glycoprotein gene family expression in different cancers. Clin Cancer Res 2004;10(24):8501-11.
18. Kovacheva M, Zepp M, Berger SM, Berger MR. Sustained conditional knockdown reveals intracellular bone sialoprotein as essential for breast cancer skeletal metastasis. Oncotarget 2014;5(14):5510-22.
19. Bellahcène A, Bonjean K, Fohr B, Fedarko NS, Robey FA, Young MF et al. Bone sialoprotein mediates human endothelial cell attachment and migration and promotes angiogenesis. Circ Res 2000;86(8):885-91.
20. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev 2008;19(5-6):333-45.
21. Kahles F, Findeisen HM, Bruemmer D. Osteopontin: A novel regulator at the cross roads of inflammation, obesity and diabetes. Mol Metab 2014;3(4):384-93.
22. Ramaiah SK, Rittling S. Pathophysiological role of osteopontin in hepatic inflammation, toxicity, and cancer. Toxicol Sci 2008;103(1):4-13.
23. Takeuchi E, Sugamoto K, Nakase T, Miyamoto T, Kaneko M, Tomita T et al. Localization and expression of osteopontin in the rotator cuff tendons in patients with calcifying tendinitis. Virchows Arch 2001;438(6):612-7.
24. Lund SA, Giachelli CM, Scatena M. The role of osteopontin in inflammatory processes. J Cell Commun Signal 2009;3(3-4):311-22.
25. Xue TC, Zhang L, Xie XY, Ge NL, Li LX, Zhang BH et al. Prognostic significance of the neutrophil-to-lymphocyte ratio in primary liver cancer: a meta-analysis. PLoS One 2014;9(5):e96072.

How to cite this article:

Hıra S, Tamam C, Demırpek U, Gem M. Evaluation of Serum Levels of Osteopontin and Bone Sialoprotein in Female Patients with Tendinopathy. J Clin Anal Med 2017;8(4): 271-4.